REMARKS

Upon entry of this amendment, claims 401, 411, 414, 416, 419, 422-424, 464-465, 469-471 and 478-480 will be pending in the application.

Claims 437 and 438 have been cancelled. Applicants reserve the right to pursue the subject matter of these claims in one or more continuing applications.

Independent Claims 401 and 411 have been amended. Claim 401 has been amended to recite that the nicotinamide is in the same culture medium as the cytokines. Support for this amendment is found throughout the specification and in Example 5. Claim 401 has also been amended to recite that the expanded CD34+ hematopoietic stem cell culture has an increased proportion of CD34+/Lin- and CD34+/CD38- cells in the expanded culture as compared to CD34+ cells cultured in the presence of cytokines and nutrients without exogenously added nicotinamide. Claims 401 and 411 have been amended to recite that IL-3 is optional. Support for this amendment is found throughout the specification and in Example 1. Claims 401 and 411 have been amended to delete "nicotinamide analog or nicotinamide derivative". Dependent claims 464, 465, 469-471 and 478-489 have been amended similarly. Claim 411 has also been amended to clarify that the cell population is "isolated". Some grammatical changes have also been made to claims 401 and 44 for clarity. No new matter is added.

Rejection Under 35 U.S.C. § 101

Claim 411 and dependent claims therefrom were rejected under 35 U.S.C. § 101 for failing to state that the composition was "isolated". Independent Claim 411 has been amended to recite that the cells are "isolated". The dependent claims necessarily contain the same limitation. This rejection is moot in view of the claim amendments and should be withdrawn.

Rejection Under 35 U.S.C. § 112, first paragraph (written description)

Various claims were rejected as failing the written description requirement for the recitation "nicotinamide analog or nicotinamide derivative". While applicants do not agree with the rejection, for the purpose of expediting prosecution of the instant case, all claims have been amended to delete the terms "nicotinamide analog or nicotinamide derivative". This rejection is moot in view of the claim amendments and should be withdrawn.

Rejections Under 35 U.S.C. § 103(a)

Claims 401, 411, 414, 416, 419, 422-424, 437, 438, 464, 465, 469-471 and 478-480 are rejected under 35 U.S.C. § 103(a) as being unpatentable over US Patent Publication No. 2002/0159984 ("Brown") in view of U.S. Patent No. 6,413,772 to Block ("Block"). Applicants traverse.

Independent Claim 401 (and the claims that depend therefrom) are amended herein to recite methods for *ex-vivo* expansion of hematopoietic stem cells by culturing the cells in a growth medium that contains cytokines for proliferation and in the same medium contains nicotinamide at a concentration of between 1.0 mM to 10 mM to inhibit differentiation such that the method of claim 401 produces an expanded CD34+ hematopoietic stem cell population with an increased proportion of CD34+/CD38- and CD34+/Lin- cells in the expanded culture as compared to CD34+ cells cultured in the presence of cytokines and nutrients without exogenously added nicotinamide. Similarly, independent claim 411 (and the claims that depend therefrom) are amended herein to recite an isolated transplantable hematopoietic cell preparation that is characterized by a greater percentage of $CD34^+/CD38^-$ and $CD34^+/Lin^-$ cells as compared to hematopoietic stem cells propagated in the presence of cytokines and nutrients without exogenously added nicotinamide.

The criteria for obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art.[1] A prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention.[2] Moreover, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one ordinary skill in the art.[3]

The combination of Brown and Block teaches away from the present invention and that one of ordinary skill in the art would not combine the teachings of Brown and Block to reach the present invention with a predictable results.

Brown does not teach or suggest using nicotinamide for producing an expanded population of CD34+ hematopoietic stem cells while inhibiting the differentiation of the

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[1] *In re Dow Chemical Co.*, 837 F.2d 469 (Fed. Cir. 1988).

[2] *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983).

[3] MPEP §2143.01, citing *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 82 USPQ2d 1385, 1396 (2007)

$CD34^+$stem cells to produce a population enriched in CD34+/CD38- and CD34+/Lin- cells in the expanded culture. Brown most certainly does not teach or suggest the claimed method or composition for expanding using nicotinamide in the range of 1.0 mM to 10 mM, as required by the instant claims. The Examiner concedes that Brown is fatally deficient in failing to disclose this range of nicotinamide concentration (see Office Action, p. 5). Rather, Brown merely lists nicotinamide, at a very low concentration (outside the claimed range) of 4 mg/L (0.33 mM), along with 44 other compounds, as a component of a culture medium called IMDM. *See*, for example, Brown at Table I, page 4.

There is not one word in Brown suggesting to the skilled artisan that nicotinamide (in the claimed concentration range) can act as an agent that maintains CD34+ hematopoietic cells in an undifferentiated state (i.e., enriched in CD34+/CD38- and CD34+/Lin- cells) while the cells are expanded in *ex vivo* culture using a serum-containing culture medium – as claimed here. To the contrary, Brown teaches away. Brown's data clearly shows that under Brown's culture conditions, when serum is present, the CD34+ population begins to differentiate and the population of CD34+/CD38- cells declines – exactly the opposite of the express recitations of the instant claims. Specifically, as Figure 3 of Brown shows, by day 14, the population of CD34+/CD38- cells has declined to less than 1X of the proportion of that undifferentiated population at day 0. Brown expressly states that this is due to differentiation of the CD34+ cell population under culture conditions when serum is present – see Example 1, specifically [¶¶106-107].

In contrast, in the instantly claimed invention, the presence of nicotinamide in serum-containing culture medium unexpectedly yielded a large enrichment for undifferentiated CD34+ hematopoietic stem cell populations that have a substantially increased cell density of undifferentiated CD34+/CD38- and CD34+/Lin- cells. See Figs, 15-16 and [¶¶173-175] and [¶¶567-568].

Further, as described previously in the record, nicotinamide concentrations of 4 mg/L nicotinamide (0.33 mM) disclosed by Brown are ineffective in inhibiting the differentiation of the $CD34^+$stem cells. *See*, 2008 Peled Declaration. In contrast, as shown in the data accompanying the 2008 Peled Declaration, using nicotinamide in the range of 1.0 mM to 10 mM, as required by the instant claims inhibited the differentiation of the $CD34^+$stem cells. *See*, December 30, 2008 Peled Declaration at pages 2-4 and at Figure 1.

Moreover, the culture conditions of Brown teach away from the use of nicotinamide to inhibit the differentiation of $CD34^+$stem cells. Specifically, Brown teaches that one of the problems to be solved in the art to improve stem cell engraftment is to prevent the differentiation of $CD34^+$ cells which typically occurs when the cells are cultured in a serum-containing medium. *See*, Brown, at page 1, paragraph [0009]. Brown solves this problem by culturing the cells in a serum-free medium. *See*, Brown, at page 3, paragraphs [0035]-[0041]. These teachings of Brown are in direct contrast to the instant invention wherein the cells require serum, nutrients and cytokines to promote proliferation but also require nicotinamide in an amount effective to inhibit differentiation (1.0 mM to 10 mM).

Block does not cure the deficiencies of Brown. To the contrary, Block teaches away. First, Block refers to the use of nicotinamide in the culture/expansion of differentiated hepatocytes – a completely different cell population that the claimed CD34+ hematopoietic stem cell population. One of ordinary skill in the art would readily recognize that the hepatocyte culture conditions described by Block would be unsuitable to the culturing conditions of $CD34^+$ hematopoietic stem cells. For example, none of the cytokines recited in instant claims are present in Block culture medium. More importantly, the ordinarily skilled artisan would not select (out of the many "ingredients" in Block's culture medium) nicotinamide for use as claimed here – to the contrary, Block teaches the exact opposite. Specifically, in Block, nicotinamide was used to maintain the differentiated state of the hepatocytes (exactly opposite to the use in the currently claimed invention). See Block, col. 8, lines 26-28 ("Nicotinamide, another HBM component, has been shown to maintain hepatocyte differentiation…"). Because Block expressly states that nicotinamide is a differentiation agent, it is not reasonable to conclude that the ordinarily skilled artisan would combine this "ingredient" at the concentration used in Block with Brown to accomplish exactly the opposite result claimed here – maintaining an undifferentiated cell population.

Further Block also directly teaches away from the use of serum in the culture – as expressly recited in the claims here. In fact, the entire focus of Block is to provide a chemically defined culture medium that is serum free. See, e.g., col. 1, lines 43-50 and col. 4, lines 8-10.

One of ordinary skill in the art combining the teachings of Brown and Block would not, and could not, reach the present invention with predictable results.

Moreover, the data presented in the 2008 Peled Declaration and the working example provided in the instant specification at Example 5 readily demonstrates that the present invention

provides unexpected and superior properties not taught or suggested by the prior art, *e.g.*, that the critical range1.0 mM to 10 mM exogenously added nicotinamide inhibits differentiation of the CD34+ hematopoietic stem cells (as evidenced by the unexpectedly and substantially increased cell density of undifferentiated CD34+/CD38- and CD34+/Lin- cells), while permitting expansion, *ex vivo*.

Applicants respectfully request reconsideration and withdrawal of the present rejection.

Claims 437, and 438 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Brown and Block and further in view of Banasik *et al.*, 1992 JBC, 1569-1575 ("Banasik"). *See*, Office Action at pages 11-12. Applicants traverse with respect to the pending claims, as amended.

As described above, Brown and Block teach away from the present invention and do not teach or suggest using nicotinamide for inhibiting the differentiation of the $CD34^+$stem cells and do not teach or suggest using the range of 1.0 mM to 10 mM, as required by the instant claims.

Banasik does not cure the deficiencies of Brown and Block. In contrast, Banasik merely discloses that benzamide, as well as nicotinamide, is an inhibitor of poly(ADP-ribose) synthetase activity. Banasik is silent with regard to the use of nicotinamide for inhibition of differentiation of $CD34^+$stem cells. Since the claims as amended recite nicotinamide only (and not analogs), the rejection is moot.

For the foregoing reasons, the claimed invention is not obvious in view of the combination of Brown, Block and Banasik. The rejection should be withdrawn.

CONCLUSION

Applicants submit that the application is in condition for allowance and such action is respectfully requested. However, if upon receipt and review of this amendment, the Examiner believes that the present application is not in condition for allowance and that changes can be suggested which would place the claims in allowable form, the Examiner is respectfully requested to call Applicant's undersigned counsel at the number provided below.

Respectfully submitted,

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